

Human Recombinant Adenosine A2A Receptor Stable Cell Line**Cat. No. M00246****Version 05282014**

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I. INTRODUCTION

Catalog Number: M00246

Cell Line Name: CHO-K1/ADORA2A/Gα15

Gene Synonyms: ADORA2A; ADORA2; RDC8; hA2AR

Expressed Gene: Genbank Accession Number NM_000675; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for ADORA2A receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 200 µg/ml Zeocin, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The adenosine receptors ADORA2A is Gs-coupled GPCRs expressed in the thymus gland, heart, lung, kidney, brain, platelets, spleen and leukocytes. ADORA2A down-regulates chemokine receptor function and inhibits platelet aggregation. ADORA2A antagonists may be useful as therapy for Parkinson's disease.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by NECA in CHO-K1/ADORA2A/Gα15 and CHO-K1/Gα15 cells

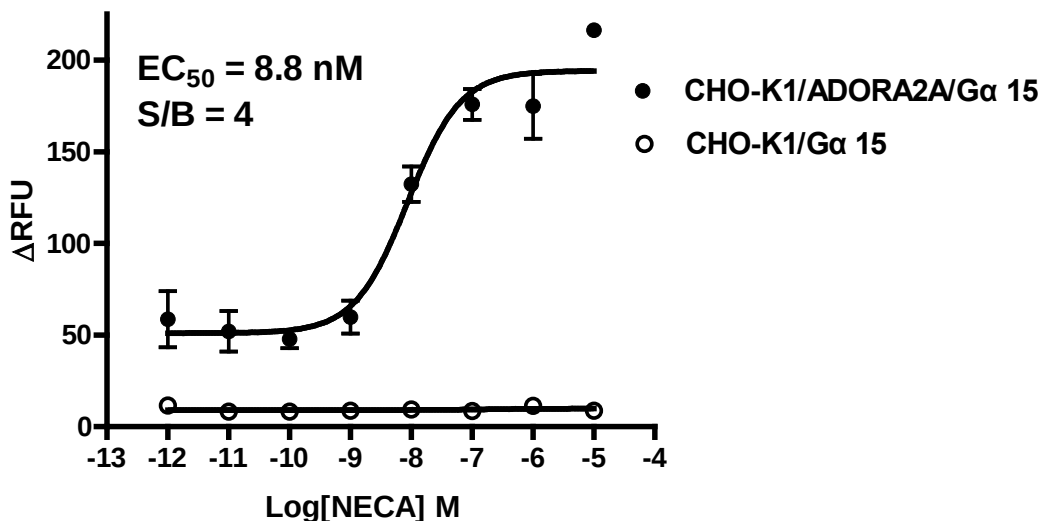


Figure 1. NECA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADORA2A/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an ADORA2A receptor agonist, NECA. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NECA (Mean ± SD, n = 2). The EC₅₀ of NECA on ADORA2A co-expressing with Gα15 in CHO-K1 cells was 8.8 nM. The S/B of NECA on ADORA2A co-expressing with Gα15 in CHO-K1 cells was 4.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

X is the logarithm of concentration. Y is the response
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Subculturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.
Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

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2. Peterfreund RA *et al.* (1996) Characterization and expression of the human A2a adenosine receptor gene. *J Neurochem.* 66(1):362-8.
3. Bosch MP *et al.* (2004) Synthesis and biological activity of new potential agonists for the human adenosine A2A receptor. *J Med Chem.* 47(16):4041-53.

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